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The primary organization of nucleosomal core particles from actively dividing cells of lily

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Nucleosomal core particles containing different variants of histones H2A and H2B were isolated from actively growing sepals of lily buds (*Lilium candida*, L.). The particles had decreased electrophoretic mobility relative to those from other sources. Comparison of the arrangement of histones along DNA in lily nucleosomes with previously obtained data for nucleosomes from yeast and animal cells, either active or repressed with regard to transcription and replication, has revealed no significant differences. The results suggest that the structure of nucleosomal core particles is highly conserved in all eukaryotes, whatever the functional state of the cell and the structure of histones H2A and H2B.

Plant histone; Nucleosomal core particle; Primary organization; (*Lilium candida*)

1. INTRODUCTION

The chromatin within nuclei of plant cells, just as in other eukaryotic cells, consists of nucleosomes containing two molecules each of histones H2A, H2B, H3 and H4 and 145 base pairs (bp) of DNA [1,2]. The primary structure and electrophoretic mobility of histones H3 and H4 from plants were shown [3–7] to be very similar to those of H3/H4 from other sources. By contrast, histones H2A and H2B in plants are represented by a number of subfractions [5–7,9] that usually have a greater molecular mass and lower electrophoretic mobility in the presence of SDS than do these histones from animals, due primarily to differences in the N-terminal parts of these molecules [6,8,9].

The present paper describes the primary organization (linear arrangement of histones along DNA) [10] in nucleosomal core particles of actively dividing somatic cells of lily.

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2. MATERIALS AND METHODS

Nuclei from actively dividing cells of lily bud sepals (*Lilium candida*, L.) were isolated as described earlier [11]. The length of buds increased from 3 to 35 mm after just 9 days of growth. Bud sepals (60 g) were powdered with dry ice. The ice was evaporated at −20°C, the pulverized material was stirred in 1.5 l of buffer 1 containing 2% Triton X-100, 0.3 M sucrose, 0.5 mM diisopropyl fluorophosphate, 1% dimethyl sulphoxide, 50 mM Tris-Cl, pH 7.6, for 12 h at 4°C, filtered through several layers of cheesecloth, and centrifuged for 10 min at 500 × *g*. The pellet was washed twice in 350 ml of the same buffer containing 1% Triton X-100, 0.4 M sucrose, centrifuged for 10 min at 500 × *g*, then suspended in 100 ml of buffer 1 containing 0.25% Triton X-100, 0.25 M sucrose, layered onto 3 vols of the same buffer containing 0.25% Triton X-100, 0.5 M sucrose, and centrifuged for 20 min at 1000 × *g*. The nuclei were washed once in 50 mM Tris-Cl, pH 7.6, centrifuged for 10 min at 500 × *g*, then washed three times in a buffer containing 0.35 M NaCl, 2 mM EDTA, 10 mM Na-phosphate, pH 6.8, and centrifuged for 10 min at 5000 × *g*.

Covalent crosslinking of histones to DNA within nuclei, isolation of crosslinked nucleosomes and analysis by two-dimensional electrophoresis were carried out as described [11]. Core particles were obtained by digestion of lily nuclei with micrococcal nuclease in 10 mM Tris-Cl, pH 8.0, 1 mM $CaCl_2$. Electrophoresis of histones in the presence of SDS [12], of DNA under denaturing conditions in the presence of 7 M urea [13], and of nucleosomes at pH 8.3 [14] has been described. Two-

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dimensional electrophoresis of lily histones was performed in the first direction in an acidic system containing 0.5% Triton X-100 [7] and in the second direction in the presence of SDS [12] and 7 M urea.

3. RESULTS AND DISCUSSION

To determine the nucleosomal core particle primary organization, histones were covalently crosslinked to DNA directly in isolated nuclei from sepals of lily buds [10,11]. To obtain nucleosomes, the conditions for preparing nucleosomes from crosslinked nuclei by micrococcal nuclease were determined by pilot experiments (fig.1A). The crosslinked core particles were isolated from hydrolysates by preparative polyacrylamide gel electrophoresis at low ionic strength [10,14]. It should be noted that under such conditions core particles from plants run much slower than core particles from rat liver, probably due to the greater size and higher positive charges of plant H2A and H2B histones. The length of DNA in isolated nucleosomes was measured as 146 ± 3 bp (fig.1B).

H2A and H2B were identified according to Spiker et al. [7] by their characteristic mobility in an acidic electrophoretic system in the presence of Triton X-100. Histone H2B was represented by about 5 subfractions, histone H2A by more than 10 subfractions and variants (see fig.2), histones H3 and H4 by only one fraction each. It appears that histone H1 is significantly depleted in lily nuclei as was also observed for many other plants [17,18].

In the process of covalent crosslinking of histones to DNA, the DNA splits at the site of crosslinking in such a manner that only the originating 5′-end of the crosslinked DNA fragment remains attached to the protein molecule. Thus the length of the 5′-end fragment determines the distance from the 5′-end of the nucleosomal DNA to the histone crosslinking site; this length is indicated in all following schemes. To measure the length of the DNA fragment crosslinked to a particular histone fraction, we used two systems of two-dimensional polyacrylamide gel electrophoresis (fig.3A,B). In either system, crosslinked DNA-histone complexes were separated in the first direction (from left to right) in accordance with the size of both the DNA and the histone molecule. In the first system, histones were then digested with Pronase directly in the gel and the liberated ^{32}P-labeled DNA fragments separated according to their size in the presence of unlabeled DNA fragments of known lengths. This system divides DNA fragments crosslinked to different fractions

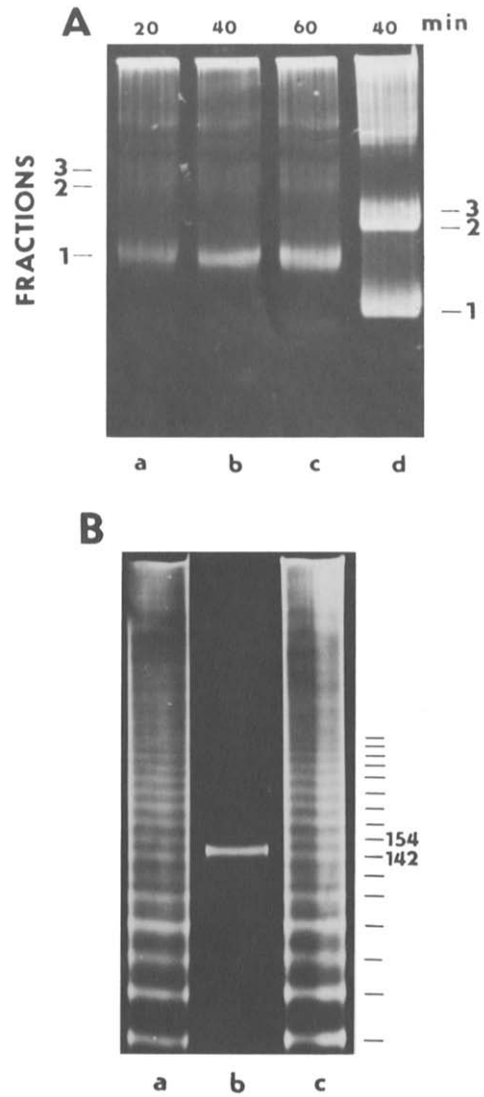

Fig.1. Electrophoretic separation of nucleosomes and nucleosomal DNA. (A) Electrophoresis in 7% polyacrylamide gel [14] of nucleosomes from micrococcal nuclease digests of nuclei from lily (lanes a–c) and rat liver (d). (1) Nucleosomal core particles; (2,3) H1-containing nucleosomes. Digestion time is given above. (B) Electrophoresis in 9% polyacrylamide gel under denaturing conditions in 7 M urea [13] of DNA fragments of known length [15,16] obtained upon digestion of rat liver nuclei by DNase I (a,c) and DNA from lily nucleosomes (b). The length of DNA fragments is indicated in nucleotides.

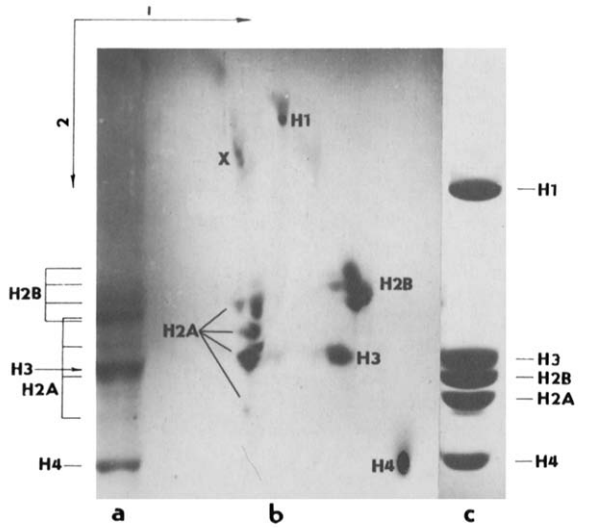

Fig.2. Two-dimensional electrophoretic separation of histones from lily nuclei (b). First direction electrophoresis was done in an acidic system in the presence of Triton X-100 [7]; second direction separation was performed in the presence of SDS [12]. In the second direction, histones from the lily core particles (a) were run in parallel with histones from lily nuclei (b) and rat liver (c). Unidentified proteins are marked with an 'X'.

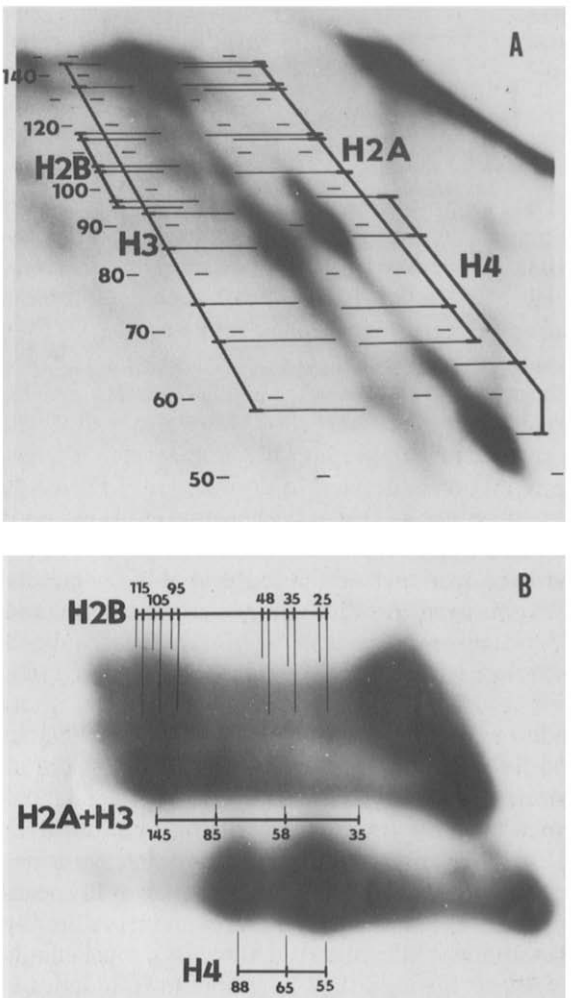

Fig.3. Two-dimensional gels revealing the arrangement of histones on nucleosomal DNA. (A) An autoradiograph of two-dimensional electrophoretic separation of ^{32}P-labeled DNA fragments crosslinked to histones within the lily core particle. Dashed lines show the positioning of 'markers' (unlabeled DNA fragments). Their exact sizes were 52, 63, 73, 83, 93, 103, 113, 124, 133 and 142 nucleotides [15,16]. Solid horizontal lines show the position on the corresponding diagonals of the 5′-end fragments of nucleosomal DNA, crosslinked to a particular histone fraction. (B) An autoradiograph of two-dimensional separation of ^{125}I-labeled histones crosslinked to DNA within the lily core particle. The size of the DNA 5′-end fragment crosslinked to histone as measured from A is given for each histone spot. The spots of uncrosslinked histones are seen in the extreme right part of the figure.

of histones into separate diagonals (fig.3A). In the second electrophoretic system, the first-dimension electrophoresis of DNA-histone complexes was followed by hydrolysis of the DNA and the liberated ^{125}I-labeled histones were then fractionated by second-dimension electrophoresis in the presence of SDS, as described in fig.2. The shift in positioning of crosslinked histones to the left from the fraction of free histones in the right-hand part of the gel in fig.3B is proportional to the size of covalently bound DNA fragments measured in fig.3A. The bulk of information about the length of crosslinked DNA is derived from fig.3. This method is more useful for determining crosslinking sites corresponding to the short DNA fragments and for separating the H2A and H2B subfractions which give the poorly resolved diagonals in fig.3A.

In fig.3 one can see the spots H4(55), H4(88), and weaker spots H4(65) and H4(98). Histone H3 gives strong spots H3(85) and H3(135,145), weaker spots H3(75) and H3(95) and scarcely seen H3(58) and H3(68). The spot (105) in the H3 diagonal may actually be H2A(105), as discussed below. Histone H2B is represented in fig.3 by two subfractions: strong spots H2B(95), H2B(105) and H2B(115) and weaker spots H2B(24,35,38,48). For histone H2A, only one subfraction is readily identifiable.

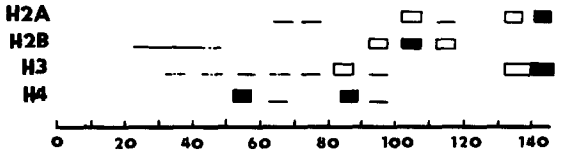

Fig.4. Localization of histone-crosslinking sites on either complementary DNA strand in the lily core particle. The crosslinking efficiencies for each histone determined from the relative intensity of each radioactive spot on autoradiographs of two-dimensional electrophoretic separations. The crosslinking sites are shown here, in order of increasing crosslinking efficiency by broken lines, solid lines, open bars, black bars. Each successive symbol indicates an increase in crosslinking efficiency of 3–5-fold over the previous symbol. Distances along each DNA strand are indicated in nucleotides from the 5′-end.

The diagonal of H2A with spots H2A(135,145) and a very weak spot H2A(118) is located between the diagonals of histones H3 and H4. Other subfractions of H2A fall on the diagonals of H2B and H3 as they have the same mobility in the first direction electrophoresis in the presence of SDS (fig.2). This could account for the presence of weak spots in the region H2B(135,145) on the H2B diagonal and for the drastically enhanced H2B(105) spot as compared with the H2B(95,115) spot (fig.3A) which is rather unusual for this histone [10,11].

Fig.4 shows a scheme for the primary organization of nucleosomal core particles from lily summarizing results of 4–5 experiments. The positioning of histone-DNA binding sites in lily sepal cells distinguished by a high mitotic index is very similar to their arrangement in transcriptionally active, replicating cells of Ehrlich ascites mouse carcinoma, rat liver, *Drosophila* embryos and yeast, and repressed cells of sea urchin sperm and chicken erythrocytes [10,11]. This result is in good agreement with the idea that the sequential arrangement of histones is highly conserved in evolution [17] and that the general pattern of nuclease digestion of nuclei originating from all three higher eukaryote kingdoms [20–23] – Zoo, Phyta and Fungi [24] – is very similar. This is in line with a finding that histones from plant and animal nucleosomal core particles seem to be interchangeable in reconstitution of the histone octamer [25–27] or core particles [28] and agrees also with the similarity in character [29] and localization [29] of histone interactions within the histone octamers in plants and animals [30].

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